

1. A method of assaying for the equilibrium interaction of a probe and an unknown target, said method comprising:
exciting a sample at with radiation, said sample comprising
at least a portion of the members of a library,
at least one probe, and
at least one fluorescent tag;
measuring the fluorescence from a subvolume of said sample; and
analyzing the fluctuations of said fluorescence.

5

10 2. The method of claim 1 further comprising
selecting additional portions of said library,
sequentially exciting an additional portion of said library with radiation;
measuring the fluorescence of a subvolume of the additional portion; and
analyzing the fluctuations of said fluorescence.

15

15 3. The method of claim 1, said sample comprises a plurality of fluorescent tags, said fluorescent tags being attached to said members.

20 4. The method of claim 1 further comprising separating at least one of the members of said portion of said library from at least one other member of said portion of said library, and repeating the method of claim 1 on said at least one separated member.

25 5. The method of claim 1, wherein said members comprise said fluorescent tag.

6. The method of claim 1, wherein said fluorescent tag is attached to said probe.

7. The method of claim 1, further comprising generating a library.

8. The method of claim 1, further comprising generating a library comprising fluorescent members.

9. The method of claim 8, wherein said generating comprises in vitro
5 translation.

10. The method of claim 1, further comprising labeling said members of said library with a fluorophore.

10 11. The method of claim 10, wherein said labeling comprises in vitro translation labeling using a fluorescent amino acid analogue, labeling by inserting a sequence for a fluorescent protein into a cDNA or post translational labeling.

15 12. The method of claim 1, wherein said members of said library comprise fluorescent proteins.

13. The method of claim 1, wherein said members of said library comprise fluorescently tagged amino acids.

20 14. The method of claim 1, wherein said members of said library comprise fluorescently labeled peptides.

25 15. The method of claim 1, wherein said sample comprises a plurality of unique probes, each unique probe comprising a unique fluorescent tag, each unique probe having a unique binding site.

16. The method of claim 1, wherein when binding of a probe and a member is present, said method further comprises identifying the member with which the probe has formed a bond.

17. The method of claim 1, wherein said sample further comprises a second
fluorescently labeled probe, said first fluorescently labeled probe and said second
fluorescently labeled probe being capable of binding to two different unique binding sites.

5 18. The method of claim 1, wherein said sample further comprises a second
probe capable of binding to a unique site on a target, said unique site being created when
said first probe binds to the target.

10 19. The method of claim 1, wherein said at least one fluorescent tag is attached
to a second probe, said second probe being capable of binding to a unique site on at least
one of a target and the first probe when said first probe is bound to the target, said unique
site being created when said first probe binds to the target.

15 20. The method of claim 19, wherein said unique site is derived from a change
in at least one of the primary, secondary and tertiary structure of at least one of the target
and the first probe.

20 21. The method of claim 19, wherein said unique site is created by the addition
of a moiety to the target.

22. The method of claim 19, wherein said unique site is created by at least one
of phosphorylation, glycosylation, alkylation, acylation, acetylation, and ubiquitination.

25 23. The method of claim 19, wherein said unique site is created by proteolysis.

24. The method of claim 19, wherein said unique site is selected from the
group consisting of a phosphotyrosine, phosphoserine, or a combination thereof.

30 25. The method of claim 1, wherein said members comprise a binding site
created by at least one of phosphorylation, glycosylation, proteolysis, and ubiquitination.

26. The method of claim 1, wherein at least one of said probe and said member is attached to a bead.

27. The method of claim 1, wherein said probe is attached to said bead and said
5 fluorescent tag is attached to said member.

28. The method of claim 1, wherein said member is attached to said bead and said fluorescent tag is attached to said probe.

10 29. The method of claim 1, wherein said analyzing comprises determining at least one of the size of the fluorescence intensity fluctuations and the duration of the correlation of the fluorescence fluctuation.

15 30. The method of claim 1, wherein said analyzing comprises determining a correlation function comprising at least one of the crosscorrelation function of said sample and an autocorrelation function of said sample.

31. The method of claim 30, wherein said analyzing further comprises determining the decay time of the correlation function.

20 32. The method of claim 30, wherein said analyzing further comprises determining the time zero value of the correlation function.

25 33. The method of claim 1, wherein said analyzing comprises at least one of a moment analysis, Fourier transform analysis, and a power spectrum analysis.

30 34. The method of claim 1, wherein when binding is present, said method further comprising determining at least one of the diffusion coefficient of a probe-member complex, the number of probe-member complexes in the sample, and the stoichiometry of the probe-member complex.

35. The method of claim 1, wherein said sample further comprises a plurality of unique probes, wherein each unique probe comprises a unique fluorophore.

36. The method of claim 1, wherein said sample further comprises a plurality 5 of different size beads, a plurality of probes and a plurality of members of said library, at least one of said probes and said members being attached to said beads.

37. The method of claim 35, wherein said members are attached to said beads and said probes comprise a fluorescent tag.

10

38. The method of claim 35, wherein said probes are attached to said beads and said members comprise a fluorescent tag.

15

39. The method of claim 1, wherein said sample further comprises a second fluorescent tag different from said first fluorescent tag.

40. The method of claim 38, wherein said first fluorescent tag is attached to said probe and said second fluorescent tag is attached to said member.

20

41. The method of claim 38, wherein said first fluorescent tag is attached to said first probe and said second fluorescent tag is attached to at least one of a second probe and a bead.

25

42. The method of claim 38, wherein said first fluorescent tag is attached to said member and said second fluorescent tag is attached to at least one of said probe and a bead.

30

43. The method of claim 38, wherein said sample further comprises a plurality of different size beads and at least one of said probe and said member is attached to said beads.

44. The method of claim 42, wherein said sample further comprises a plurality of unique probes, each unique probe being attached to a different size bead.

45. The method of claim 42, wherein said first fluorescent tag is attached to
5 said probe and said second fluorescent tag is attached to said unknown target.

46. The method of claim 42, wherein said first fluorescent tag is attached to said first probe and said second fluorescent tag is attached to at least one of a second probe and said beads.

10

47. The method of claim 1, wherein said sample comprises a crosslinking agent.

48. The method of claim 1, wherein at least one of said probe, said member,
15 and said fluorescent tag comprises a crosslinking agent.

49. The method of claim 1, further comprising determining at least one of a true autocorrelation function and a true crosscorrelation function of said sample.

20

50. The method of claim 1 further comprising flowing said sample through a sample chamber.

51. The method of claim 1, wherein said method is automated.

25

52. A method of assaying for the equilibrium interaction of a probe and an unknown target, said method comprising:

exciting a sample with radiation, said sample comprising

at least one unknown target,

at least one probe, and

30 at least one fluorescent tag;

measuring the fluorescence from a subvolume of said sample; and
analyzing the fluctuations of said fluorescence.

53. The method of claim 52, wherein at least one of said probe and said
5 unknown target comprises said fluorescent tag.

54. The method of claim 52, wherein said fluorescent tag is attached to said
probe.

10 55. The method of claim 52, wherein said fluorescent tag is attached to said
unknown target.

56. The method of claim 52, wherein when binding is present, said method
further comprises identifying the unknown target with which the probe has formed a bond.

15 57. The method of claim 52, wherein said unknown target comprises a product
resulting from pathogen infection.

58. The method of claim 52, wherein said unknown target comprises a toxin.

20 59. A method of assaying for a pathogen in a sample, said method comprising:
exciting a sample with radiation, said sample comprising
at least one pathogen;
at least one probe, and
25 at least one fluorescent tag;
measuring the fluorescence from a subvolume of said sample; and
analyzing the fluctuations of said fluorescence.

30 60. A method of assaying for the presence of a pathogen component in a
sample, said method comprising:
exciting a sample with radiation, said sample comprising
at least one probe capable of binding a predetermined pathogen
component, and

at least one fluorescent tag;
measuring the fluorescence from a subvolume of said sample;
analyzing the fluctuations of said fluorescence; and
determining the presence or absence of said pathogen component.

5

61. The method of claim 60, further comprising identifying said pathogen.

62. The method of claim 60, wherein said sample comprises a plurality of unique fluorescently tagged probes, each unique probe comprising a unique fluorophore,
10 each unique probe being capable of binding to a unique pathogen component.

63. The method of claim 60, wherein said sample further comprises a second fluorescent tag comprising a fluorophore different from the fluorophore of said first fluorescent tag.

15

64. The method of claim 60, wherein said analyzing comprises at least one of determining the crosscorrelation function of said sample and determining the autocorrelation function of said sample.

20 65. The method of claim 60, wherein said pathogen component comprises a bacterium.

66. The method of claim 60, wherein said pathogen component comprises a virus.

25

67. The method of claim 60, wherein said pathogen component is selected from the group consisting of pathogen, pathogen fragment, pathogen nucleic acid, pathogen protein, pathogen carbohydrate, and combinations thereof.

68. The method of claim 60, wherein said pathogen component is selected from the group consisting of pathogen spore, pathogen toxin, metabolic product of pathogen, and combinations thereof.

5 69. The method of claim 60, wherein said pathogen component is a pathogen and said probe is capable of binding to a pathogen.

70. A method of assaying for the presence of a toxin in a sample, said method comprising:

10 exciting a sample with radiation, said sample comprising
at least one probe capable of binding a predetermined toxin, and
at least one fluorescent tag;
measuring the fluorescence from a subvolume of said sample;
analyzing the fluctuations of said fluorescence; and
15 determining the presence or absence of said toxin.

71. The method of claim 70, wherein said toxin is ricin.

72. The method of claim 71, wherein said probe and said fluorescent tag
20 comprise fluoresently tagged human serum albumin galactose.

73 The method of claim 72 wherein said probe and said fluorescent tag
comprise fluoresently tagged human serum albumin galactose.

25 74. A method of identifying a probe capable of binding to a known pathogen,
said method comprising:

- a. exciting a sample with radiation, said sample comprising at least one known pathogen, at least one probe, and at least one fluorescent tag;
- c. measuring the fluorescence emitted by the sample; and
- 30 d. analyzing the fluctuations of said fluorescence.

75. A kit comprising:
a first probe comprising
ricin,
a fluorescent tag attached to said ricin; and
5 a second probe bound to said first probe, said second probe being adapted
to bind ricin.

76. The kit of claim 75, wherein said second probe comprises human serum
albumin galactose.

10 77. The kit of claim 75, further comprising a second fluorescent tag.

78. The kit of claim 75, wherein said second fluorescent tag is attached to said
second probe.

15 79. A method of assaying for the presence of molecular interactions of a probe
and a target, said method comprising

- a. exciting a sample with radiation, said sample comprising
 - i. a plurality of unique mass adding components each unique mass adding component having a unique mass,
 - ii. a plurality of targets,
 - iii. a plurality of fluorescent tags, and
 - iv. a plurality of probes; and
- b. measuring the fluorescence emitted by the sample; and
- c. analyzing the fluctuations of said fluorescence.

20 80. The method of claim 79, wherein said fluorescent tags are attached to said
mass adding component.

25 30 81. The method of claim 79, wherein said fluorescent tags are attached to said
probes.

82. The method of claim 79, wherein said fluorescent tags are attached to said targets.

83. The method of claim 79, wherein said probes are attached to said mass
5 adding component.

84. The method of claim 79, wherein said fluorescent tags are attached to said probes and said probes are attached to said mass adding component.

10 85. The method of claim 79, wherein said fluorescent tags are attached to said mass adding component and said probes are attached to said mass adding component.

86. The method of claim 79, wherein said plurality of fluorescent tags comprise a plurality of unique fluorescent tags.

15 87. A kit comprising:
a plurality of unique beads, each unique bead having a different size;
a plurality of probes adapted to bind to a unique target, said probes being attached to said beads; and
20 a plurality of fluorescent tags.

88. The kit of claim 87, wherein said fluorescent tags comprise unique fluorophores.

25 89. The kit of claim 87, further comprising a second probe.

90. The kit of claim 87, wherein said fluorescent tags are attached to said second probe.

30 91. The kit of claim 87, wherein said fluorescent tags are attached to at least one of said beads and said probes.

92. A method of determining a true correlation function of a sample, the method comprising obtaining a measured correlation function of the sample from a fluorescence correlation spectroscopy instrument and applying a correction algorithm to
5 the measured correlation function.

93. The method of claim 92 wherein the spectroscopy instrument includes an excitation source, a first detector, and a second detector.

10 94. The method of claim 92 wherein the measured correlation function is an autocorrelation function.

95. The method of claim 92 wherein the measured correlation function is a crosscorrelation function.

15 96. The method of claim 92 wherein the correction algorithm adjusts the measured correlation function based on a bleed through coefficient.

97. The method of claim 96 wherein the correction algorithm is further based
20 on a first average of the fluorescence intensities measured at the first detector and a second average of the fluorescence intensities measured at the second detector.

98. The method of claim 92, wherein said sample comprises at least a portion of the members of a library, a pathogen, a toxin or a combination thereof.

25 99. A method of determining a true autocorrelation function of a sample, the method comprising:

obtaining a first measured autocorrelation function of the sample from a first detector of a fluorescence correlation spectroscopy instrument;

30 obtaining a second measured autocorrelation function of the sample from a second detector of the instrument;

obtaining a measured crosscorrelation function between the first detector and the second detector of the instrument; and

determining the true autocorrelation function of the fluorescence measured at the first detector.

5

100. A method of determining a true crosscorrelation function of a sample, the method comprising:

obtaining a first measured correlation function of the sample from a first detector of a fluorescence correlation spectroscopy instrument;

10 obtaining a second measured correlation function of the sample from a second detector of the instrument;

obtaining a measured crosscorrelation function between the first detector and the second detector of the instrument;

determining a true crosscorrelation function.

15

101. An article of manufacture comprising a computer readable medium having stored therein a computer program for determining a true correlation function of a sample, the computer program comprising:

20 a first code segment for obtaining a measured correlation function of the sample; and

a second code segment for applying a correction algorithm to the measured correlation function.

102. The article of claim 101 wherein the measured correlation function is an
25 autocorrelation function.

103. The article of claim 101 wherein the measured correlation function is a crosscorrelation function.

104. The article of claim 101 wherein the correction algorithm adjusts the measured correlation function based on a crosstalk parameter between the first and the second detectors.

5 105. The article of claim 104 wherein the correction algorithm is further based on a first average of the fluorescence intensities measured at the first detector and a second average of the fluorescence intensities measured at the second detector.

10 106. An article of manufacture comprising a computer readable medium having stored therein a computer program for determining a true correlation function of a sample, the computer program comprising:

15 a first code segment for obtaining a first measured autocorrelation function of the sample from a first detector of a fluorescence correlation spectroscopy instrument, a second measured autocorrelation function of the sample from a second detector of the instrument, and a measured crosscorrelation function between the first detector and the second detector of the instrument;

a second code segment for determining the true autocorrelation function of the fluorescence measured at the first detector.

20 107. A system for determining a true correlation function of a sample, the system comprising a memory device for storing information related to the sample and a processor programmed with instruction to obtain a measured correlation function of the sample from a fluorescence correlation spectroscopy instrument and apply a correction algorithm to the measured correlation function.

25 108. A fluorescence correlation spectroscopy instrument for determining a true correlation function of a sample, the instrument comprising:

an excitation source;

a first detector and a second detector for measuring fluorescence of the

30 sample;

a memory device for storing information related to the sample; and

a processor programmed with instruction to obtain a measured correlation function of the sample from a fluorescence correlation spectroscopy instrument and apply a correction algorithm to the measured correlation function.

5 109. A method of determining a true fluorescence intensity of a sample, the method comprising obtaining a measured fluorescence intensity of the sample from a first detector of a fluorescence correlation spectroscopy and applying a correction algorithm to the measured fluorescence intensity.

10 110. The method of claim 109 wherein the correction algorithm adjusts the measured fluorescence intensity based on a bleed-through coefficient between the first detector and a second detector of a fluorescence correlation spectroscopy.

15 111. The method of claim 110 wherein the correction algorithm is further based on a second measured fluorescence intensity of the sample from the second detector.

112. A method of determining a true fluorescence intensity of a sample, the method comprising:

20 measuring a first fluorescence intensity of the sample at a first detector of a fluorescence correlation spectroscopy instrument and a second fluorescence intensity the sample at a second detector of a fluorescence correlation spectroscopy instrument; and

25 determining at least one of a true fluorescence intensity of the fluorescence measured at the first detector and the true fluorescence intensity of the fluorescence measured at the second detector.

113. The method of claim 112 further comprising generating a true autocorrelation curve, based on the first true fluorescence intensity.

30 114. The method of claim 112 further comprising generating a true crosscorrelation curve, based on the first and second true fluorescence intensities.

115. An article of manufacture comprising a computer readable medium having stored therein a computer program for determining a true fluorescence intensity of a sample measured by a fluorescence correlation spectroscopy instrument.

5

116. A fluorescence correlation spectroscopy instrument for determining a true fluorescence intensity of a sample, the instrument comprising:

an excitation source;

a first detector and a second detector for detecting a first measured

10 fluorescence and a second measured fluorescence of the sample;

a memory device for storing computer code; and

a processor for executing the computer code to obtain the true fluorescence intensity, based on the first and the second measured fluorescence.

15

117. The instrument of claim 116 wherein the memory device is an EPROM.